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# STUDY OF PRODUCTION AND TOXICITY OF CULTURED *GAMBIERDISCUS TOXICUS*

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## Abstract

*Gambierdiscus toxicus* has been described as the chief producer of ciguateric toxins: ciguatoxin (CTX) and maitotoxin (MTX). Dr. R. Bagnis (Malardé Institute, Tahiti) provided us with a strain of this benthic dinoflagellate in 1981 to study its cytological, physiological, and toxicological characteristics.

The growth of *G. toxicus* has been studied under various chemical and physical conditions enabling us to define optimal culture conditions. Since then, we have improved the cultivation procedure and have obtained large scale cultures of some clones and strains in different culture media.

All the experiments showed that *G. toxicus* has complex nutritive requirements and a large inertia to response at some non-drastic environmental variations. Increased growth rates were observed when cultures were treated with antibiotics.

The classical extraction procedure of the toxins has been adapted to our algal material. The method was simplified and resulted in enhanced toxinic yield.

Our principal results have demonstrated that *G. toxicus* in culture remained very toxic; 600 to 2000 cells were sufficient to kill a 20 g female mouse within 24 hours (MLD = 1–3 mg/kg). No variation of the degree of toxicity has been observed for three years.

A linear relationship between the number of *G. toxicus* cells (Coulter counted) and the weight of corresponding algal pellet has been found. This leads to an easier evaluation of the quantity of toxic extract required to calculate the minimum lethal dose (MLD).

The extraction procedure results in two toxic fractions: a water-soluble (MTX-like) and a lipid-soluble (CTX-like). The latter corresponds to 10 to 25% of the total toxicity.

A dose-time to death curve has been established with our CTX-like extracts.

## Introduction

Most tropical and intertropical coral reef seas contain the benthic dinoflagellate, *Gambierdiscus toxicus.* From wild *G. toxicus,* Yasumoto *et al.* (1977) extracted the two main toxins involved in ciguatera, ciguatoxin (CTX) and maitotoxin (MTX), which are transmitted to fish through the marine food chain (Taylor, 1979; Bagnis, 1981; Shimizu *et al.,* 1982; Withers, 1982). Although *G. toxicus* was the first dinoflagellate to be linked to the genesis of ciguateric toxins (Bagnis *et al.,* 1977), other toxic dinoflagellates, which are also potential sources of ciguateric toxins, have been isolated from ciguateric areas: *Prorocentrum lima* in the Pacific Ocean (Yasumoto *et al.,* 1984); and *Prorocentrum mexicanum* in the Atlantic Ocean (Tindall *et al.,* 1984). A number of dinoflagellates associated with the benthic community of coral reefs may contribute to the complex syndrome of ciguatera fish poisoning (Fukuyo, 1981;

Nakajima *et al.*, 1981; Steidinger and Baden, 1984), the biogenesis of which remains to be clarified.

We adapted a strain of *G. toxicus* from the Gambier Islands (kindly provided by Dr. R. Bagnis) to culture conditions in our laboratory and obtained mass cultures in order to study the main physiological, cytological, and ultrastructural characteristics of this dinoflagellate (Durand, 1984). We also established the toxicity of the culture under varying environmental conditions. The aim of this paper is to describe the main characteristics of cultured *G. toxicus*.

## Culture Conditions and Physiology of *G. toxicus*

### *Temperature*

The temperature which supported optimum growth for our strains was 26 ± 1°C. Temperature over 30°C quickly killed the cells, and little growth was observed below 22°C. The temperature range for optimum growth was limited, probably due to the original collection site of the strains (the Gambier Islands), where little variation in seawater temperature occurs. Thus, our *G. toxicus* strains could be different from those found by Besada *et al.* (1982) in the Windley Keys (Florida), providing evidence of two distinct ecotypes.

However, after numerous transfers over one year, we obtained a strain of *G. toxicus* with satisfactory growth at 20°C.

### *Light*

By modifying light intensity and photoperiod we demonstrated that the growth rate of *G. toxicus* was largely influenced by the total amount of illumination received per day (Durand and Puiseux-Dao, 1985). The best conditions were a light intensity of 10 $W \cdot m^{-2}$ (tubes: Philips TL 65W and Mazda Fluor TF 65W) and a light/dark cycle of 10 h/14 h. Only a few divisions of *G. toxicus* were recorded under continuous light, suggesting that *G. toxicus,* like most dinoflagellates (Loeblich, 1966), has alternating light/dark dependence for division. Preliminary experiments showed that *G. toxicus* cultures can be synchronized by modifying the light/dark alternation as described for *Amphidinium carterae* (Galleron, 1976). The low light tolerance of *G. toxicus* could be related to its epiphytic habits (macroalgal substrates) in nature.

*G. toxicus* exhibited horizontal phototactic migrations at the bottom of the culture flasks in response to light intensity. Cells moved toward the light source in low-light conditions and moved in the opposite direction under high-light intensity. This behavior of *G. toxicus* in culture demonstrates its great sensitivity to light. We never observed eyespot or stigma structures with transmission optical studies.

### *Culture flasks, maintenance of the cultures, cell counting*

Clonal cultures were initiated by isolating a single cell under the microscope.

Stock cultures were maintained in 250 ml Erlenmeyer flasks. Experiments were conducted in 125 or 250 ml flasks (initial inoculate: 30–50 cells/ml) containing 70–80 and 150–170 ml of medium. For large cultures, *G. toxicus* was cultivated in 1 liter Fernbach flasks or 2 l Erlenmeyer flasks, since we were able to obtain a greater biomass production of this benthic dinoflagellate in these vessels than in large glass carboys. The cultures were harvested three or four weeks after the initial inoculation. At that time, *G. toxicus* cells overloaded the bottom of the flasks forming a unique algal

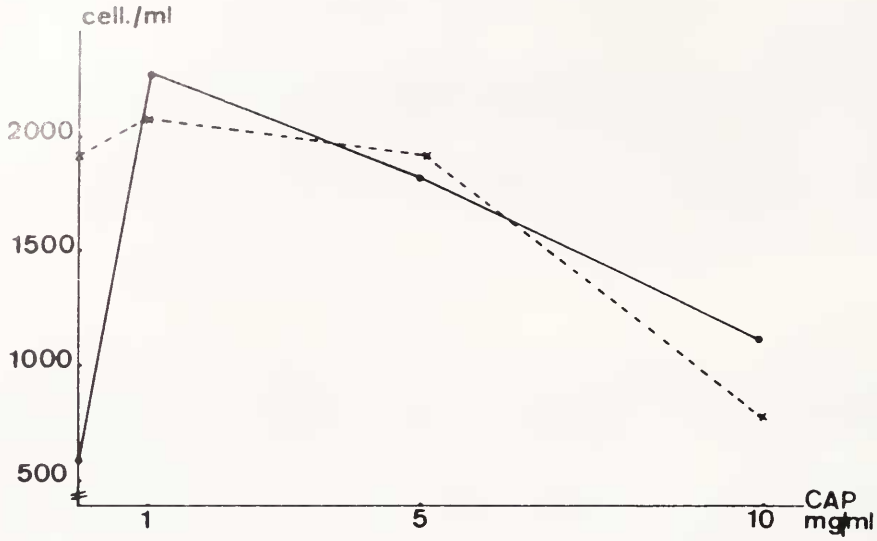



FIGURE 1. Effect of chloramphenicol (CAP) on *Gambierdiscus toxicus* growth in MPP. – – – Strain previously treated with CAP. —— Strain without previous CAP treatment.

layer. Cell density reached 4000 cells/ml (algal biomass equals 2 to $4 \cdot 10^6$ cells/1 liter Fernbach flask). Agitation of the cultures inhibited *G. toxicus* growth.

Just prior to stationary growth, cultures were harvested and washed by centrifugation (1500 × *g*, 5 min).

*Influence of bacteria on* G. toxicus *growth*

Decreasing the bacterial flora improved the growth of *G. toxicus* cultures in our laboratory, from 500–1000 cells/ml final algal yield, to 4000 cells/ml. We used two methods for this purpose: serial washing in sterile medium and antibiotic treatment. To select the most efficient antibiotics, we tested the cultures with antibiograms (multidisk Sobioda 12GR422), then treated the cultures with either a large dose of antibiotics (5–20 mg/l) for a few hours in darkness (after which the drug was washed out), or with a smaller amount (0.1–1 mg/l) added directly to the culture medium. In the first case, bacterial spore germination was promoted by adding 0.1% neopeptone one or two days prior to treatment. The best results were obtained with rifampicine, minocycline, and chloramphenicol at 5 mg/l for 24 h. (See Fig. 1 for an example of the effect of chloramphenicol on *G. toxicus*.) The number of bacterial species was decreased, from more than 50 at the origin, to 3–5. We obtained a batch in which no bacteria could be detected during two months of growth. Presently, the use of chloramphenicol (5 mg/l) results in less than one bacterium per *G. toxicus* cell. Such cultures produce only a small amount of mucilage relative to contaminated cultures.

*Culture media and nutrient supplementation assays*

Adjusting media to study the nutritional requirements of *G. toxicus* and to enhance its growth was difficult because of its slow response to environmental modifi-

cations. In addition, this large dinoflagellate showed a slow but efficient ability to adapt to various conditions.

We tried to cultivate *G. toxicus* in two different media, enriched seawater and artificial medium.

*Seawater media. G. toxicus* growth in seawater (from Banyuls/sur Mer, France) was slow (generation time: more than six days). For two months, the cells became progressively less pigmented and died.

In Lateur medium (Lateur, 1963: autoclaved seawater supplemented with $NaNO_3$, $Na_2HPO_4$, and soil extract), the growth remained slow (generation time: about five to six days) but the cultures did not degenerate.

One of the first media (Hurtel *et al.*, 1979) described for *G. toxicus* culturing was MPP, according to Provasoli and Pintner (Provasoli, 1958: autoclaved seawater supplemented with various mineral compounds and vitamins). In this medium, *G. toxicus* growth was satisfactory (division time: about three days). However, growth improved when we decreased the amount of enrichment solution (ES) from 2% to 1.5% and sometimes even 1%. When Tris (5 mg/l) was added, pH was adjusted to 8.15. No modification of *G. toxicus* growth was detected in cultures not supplemented with vitamins for six months.

*Artificial media.* The first cultures that we grew in ESAW medium (Harrison *et al.*, 1980) showed good growth for eight months and then degenerated suddenly, as reported by Carlson *et al.* (1984). Similar results ensued from a second attempt of cultures transferred in ESAW. Little amelioration of the algal growth could be obtained in this medium, even by removing NaF, silica, and SrCl or by the use of ES supplementation of MPP. Thus, ESAW is not suitable for our *G. toxicus* cultures.

In Shepard's artificial medium (MS: Shepard, 1969), *G. toxicus* growth was slow and irregular for many months (division time: five to eight days). No promotion of growth was obtained by modifying the salinity or the phosphorus concentration. In contrast, some growth stimulation occurred with the addition of 0.5% of ES (from MPP). At the moment, a strain that we progressively adapted to MS medium shows good growth (division time: four to five days) in this medium.

At present, large cultures of *G. toxicus* grow with a division time of two to four days in a mixture of MPP and MS (1:1).

*Nutrient supplementation assays.* For cultures grown in MPP medium, the following nutrient supplements did not result in a significant and reproducible improvement of culture yield: phosphates (0.1–1 m*M*), carbonates (0.5 m*M*), $Ca^{2+}$, $Mg^{2+}$, $K^+$ (0.1–1 m*M*), and glucose (1 g/l).

We tested various nitrogen sources to evaluate the nitrogen requirements of *G. toxicus*. The results are summarized below:

(1) *Mineral nitrogen:* a weak temporary increase of the division rate of *G. toxicus* was obtained by adding $NH_4Cl$ (below 0.5 m*M*) and $NaNO_3$ (5 m*M*), but these salts became toxic after a few days.

(2) *Organic nitrogen:* no amino acids tested (0.1 m*M*) stimulated *G. toxicus* growth. Addition of urea resulted in an increase of cell yield as shown in Figure 2: after one week, *G. toxicus* growth was enhanced by 0.5 m*M* urea, but this concentration became toxic; after three weeks, the largest biomass was obtained with 0.1 m*M* urea. We noticed that this stimulatory effect was more efficient while the cells were in active growth phase, suggesting that the algal metabolism must be high for urea to be used by *G. toxicus*.

(3) *Soil extract:* a variable but constant improvement of *G. toxicus* cultures was obtained by adding soil extract to MPP. We saw the following increases of the algal

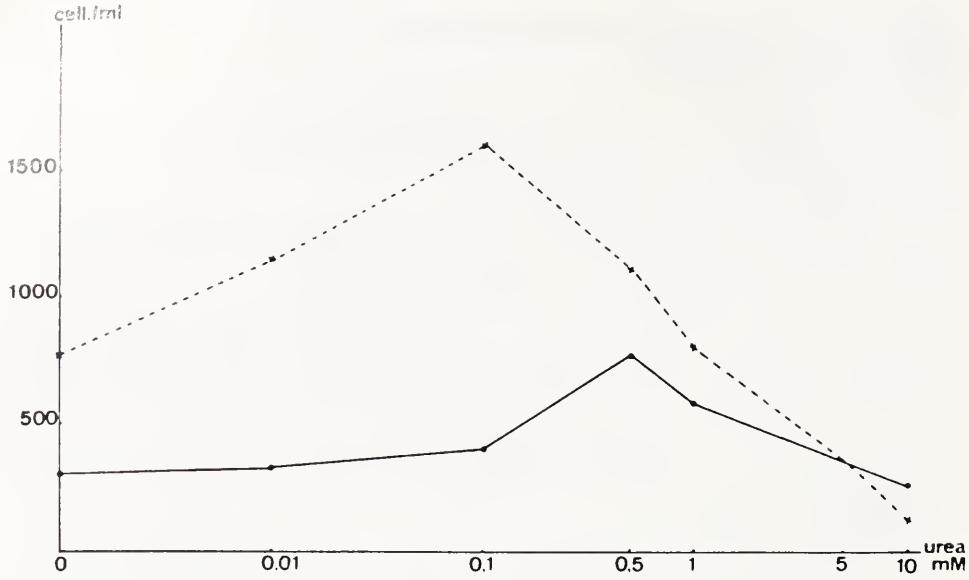



FIGURE 2. Effects of urea adjunction on *Gambierdiscus toxicus* growth in MPP medium. —— $t_0 + 7$ days. - - - $t_0 + 21$ days.

biomass 20 days after inoculation: ×1.8 for 1 g/l soil extract, ×2.4 for 2 g/l, ×2 for 4 g/l (toxic effect at higher doses). Other workers also obtained this stimulatory effect of soil extract (Carlson *et al.,* 1984; Yasumoto *et al.,* 1984).

(4) *Dilution of MPP:* diluting MPP with 5% distilled water stimulated *G. toxicus* growth (×1.5). This could be related to both a decrease of salinity and a dilution of toxic nutrients.

All the assays concerning the nutritional requirements for *G. toxicus* should be done again in MS medium (with the strain adapted to this medium) which has a well-defined chemical composition in contrast to seawater. *G. toxicus* possesses a complex physiology, and its study is complicated by the slow division rate and large size of this dinoflagellate, which allow slow responses to non-lethal variations of environmental parameters. In addition, analysis must consider the fact that cultured algae may present various adaptative behaviors, explaining the differences observed by different workers.

## Pigment Composition

Studies to determine the pigment composition of *G. toxicus* showed that this dinoflagellate contains unusual pigments (Durand and Berkaloff, 1985): it contains peridinin as the major carotenoid, and both chlorophylls $c_1$ and $c_2$. Peridinin is usually found only in association with chlorophyll $c_2$ (Jeffrey, 1976), and such pigment composition has been documented only in *Prorocentrum cassubicum.*

## Morphological and Structural Features of *G. toxicus*

We observed cultured *G. toxicus* with both light and electron microscopes (transmission TEM and scanning SEM). A Zeiss standard WL microscope equipped with Nomarski optics was used for light microscopy.

For scanning electron microscopy, *G. toxicus* algal cultures were first pre-fixed by osmium vapors for 10 min, and then fixed by adding osmium (1% for 1 h at room temperature) to the culture medium. After centrifugation, the cell pellet was rinsed with distilled water, dehydrated in an acetone series, and dried using the $CO_2$ critical-point method. The cells were then coated with gold and examined in a Cambridge 600 SEM.

In the cultures, as a function of medium composition and light conditions, different morphological aspects of *G. toxicus* could be observed: typical armored cells, deformed cells, small motile cells and cysts.

*Typical cells*

Figures 3a and b show typical *G. toxicus* cells. SEM observations were made to define accurately the thecal plate features and ornamentation of this dinoflagellate (Figs. 4a, b). Morphology and thecal plate organization were similar to that reported for wild cells by Adachi and Fukuyo (1979) and by Besada *et al.* (1982). The mean dorso-ventral diameter, for our cells, was 70 $\mu$m, with a range of 60–90 $\mu$m (30–50 $\mu$m for the vertical diameter). *G. toxicus* possesses the two typical flagella of dinoflagellates: a longitudinal one beating in the culture medium (about 50 $\mu$m length; see Fig. 3a) and a transversal one. The latter is located in the cingulum and has regular, jerky movements that substantially mixes the medium surrounding the cell.

Cell coloration varied, depending on light intensity and culture medium: *G. toxicus* cells appeared orange-brown in seawater, light brown in MPP, and dark brown in MS.

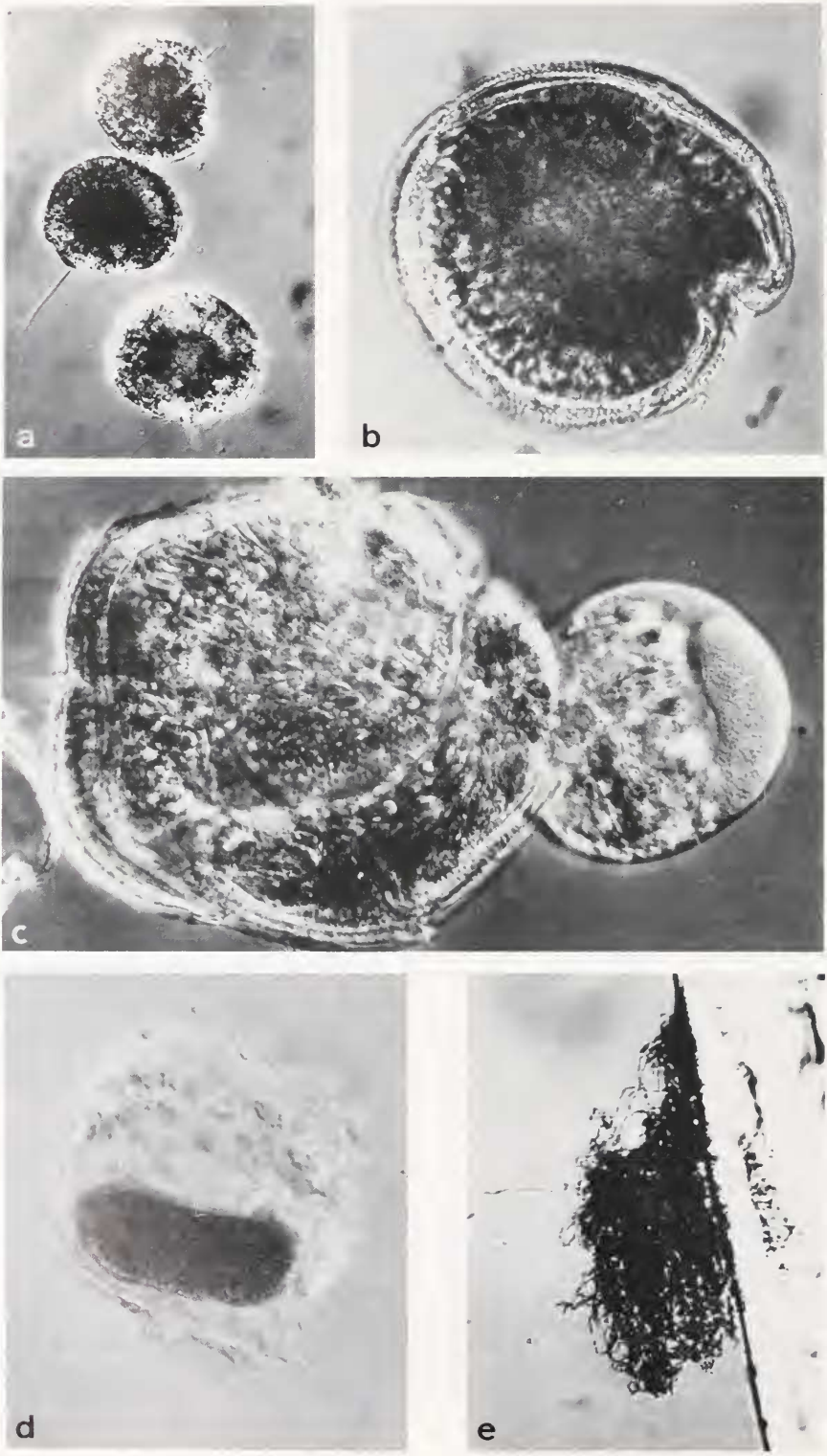
*Theca.* The *G. toxicus* cell covering consists of a thick theca (about 2 $\mu$m as seen in TEM), or amphiesma (Loeblich, 1970), which limits the cytoplasm. This covering is composed of an outer membrane, a well-developed plate layer, a dense pellicle, and a double membrane. The presence of the pellicle has been described by Morill and Loeblich (1981), who used chloral hydratehydriodic acid-iodine staining. This theca is perforated with numerous trichocystic pores.

*Nucleus.* The *G. toxicus* nucleus is large (30 $\mu$m; see Fig. 3d). It is easily ejected, intact, when the cell is pressed between a slide and a coverslip (Fig. 3c). It contains numerous permanently condensed chromosomes (Fig. 3e) and a large amount of DNA (162 pg/cell). Various nuclear stains revealed, in addition to this dinocaryon, numerous unusual structures that we called "pseudo-nuclear" vesicles: they possess nuclear features as shown by both cytochemical and electron microscopic studies (Durand *et al.*, 1986).

*Cytoplasmic features. G. toxicus* cytoplasm is very dense and contains the usual organelles found in dinoflagellates, including hundreds of trichocysts. When dehydrated (under the microscope, for instance), one or two brighter areas often appeared (Fig. 4f). They could be linked to the pusule.

*Deformed cells*

Cells having abnormal morphological aspects appeared in the cultures under some conditions (Durand and Puiseux-Dao, 1985). The morphology of these cells was deformed in comparison to typical cells: in apical view, their shape was embossed instead of circular; in a lateral view, they were round instead of flattened. The thecal-plate feature of teratogenous forms was completely disorganized as seen by SEM (Fig. 4c). Their size was large (80–100 $\mu$m), their motility reduced, and they did not stick to the bottom of the flasks. They were capable of mitosis, so they cannot be a sexual stage of *G. toxicus*.


a
b
c
d
e

Analysis of the conditions of the appearance and evolution of deformed cells in *G. toxicus* populations suggested two sources: culture-medium composition and photoperiod.

### *Small motile cells and cysts*

Cells that had abandoned their theca (ecdysis; Fig. 4d) were often observed. Some small, motile, and apparently naked cells were occasionally visible in aged cultures (Fig. 4e). They could correspond to the cells released by ecdysis described above and be a survival behavior as described for other dinoflagellates (Walker, 1982), or to a stage of hypnoid cyst formation defined by Anderson *et al.* (1978). According to these workers, the structure shown in Figures 4g and h (same cell, two different foci) would represent another type of temporary cyst (coccoid). They were detected only once, after increasing the temperature of the culture to over 30°C.

Some large (over 100 $\mu$m), dark, and non-motile cells which could be interpreted as hypnocysts, were occasionally observed. Also observed (mostly in seawater) were paired cells which could be interpreted as fused gametes. These observations suggest that sexual reproduction might occur in *G. toxicus* cultures, but this hypothesis needs to be confirmed by other observations and studies.

## TOXICITY OF *G. TOXICUS* CULTURES

*G. toxicus* toxicity was studied to evaluate the influence of culturing on its ability to synthetize toxic compounds. After verifying that *G. toxicus* was toxic, we tried to improve the toxin extraction procedure; finally, we tested a few parameters which could modify the toxin production of this dinoflagellate.

### *Evaluation of toxicity*

Toxicity was evaluated using the mouse bioassay described by Yasumoto *et al.* (1979). Toxic extracts were dried under a nitrogen stream to eliminate any trace of solvent. Each extract was emulsified in 0.4 ml of a Tween 60 solution and injected intraperitoneally into a 19–21 g mouse (Swiss female). Several doses were tested (2 mice per dose) to determine the minimal lethal dose (MLD). MLD is defined as the minimal amount of extract that causes death for a 20 g mouse within 24 h (expressed in mg/kg). To simplify comparison between the toxicity of different extracts, lethality is expressed in mouse-units (one MU equals MLD ÷ 50), and total lethality is expressed as the number of cells per mouse unit.

### *Toxin extraction procedure*

First, we extracted ciguatoxins by the classic procedure of Yasumoto *et al.* (1979) and Bagnis *et al.* (1980). This procedure was inadequate to our algal material, so we studied the yield of each step of the protocol and modified them progressively. These

FIGURE 3. *Gambierdiscus toxicus* cells. (a) Three typical *G. toxicus* cells (microcinematography, see the longitudinal flagellum), 320×. (b) A typical *G. toxicus* cell, 900×. (c) *G. toxicus* nucleus (Nu) ejected with the cytoplasm while pressing the cell between slide and coverslip, 1150×. (d) A depigmented *G. toxicus* cell after Feulgen's staining, 800×. (e) Squash of *G. toxicus* after Feulgen's staining: view of the chromosomes, 2600×.

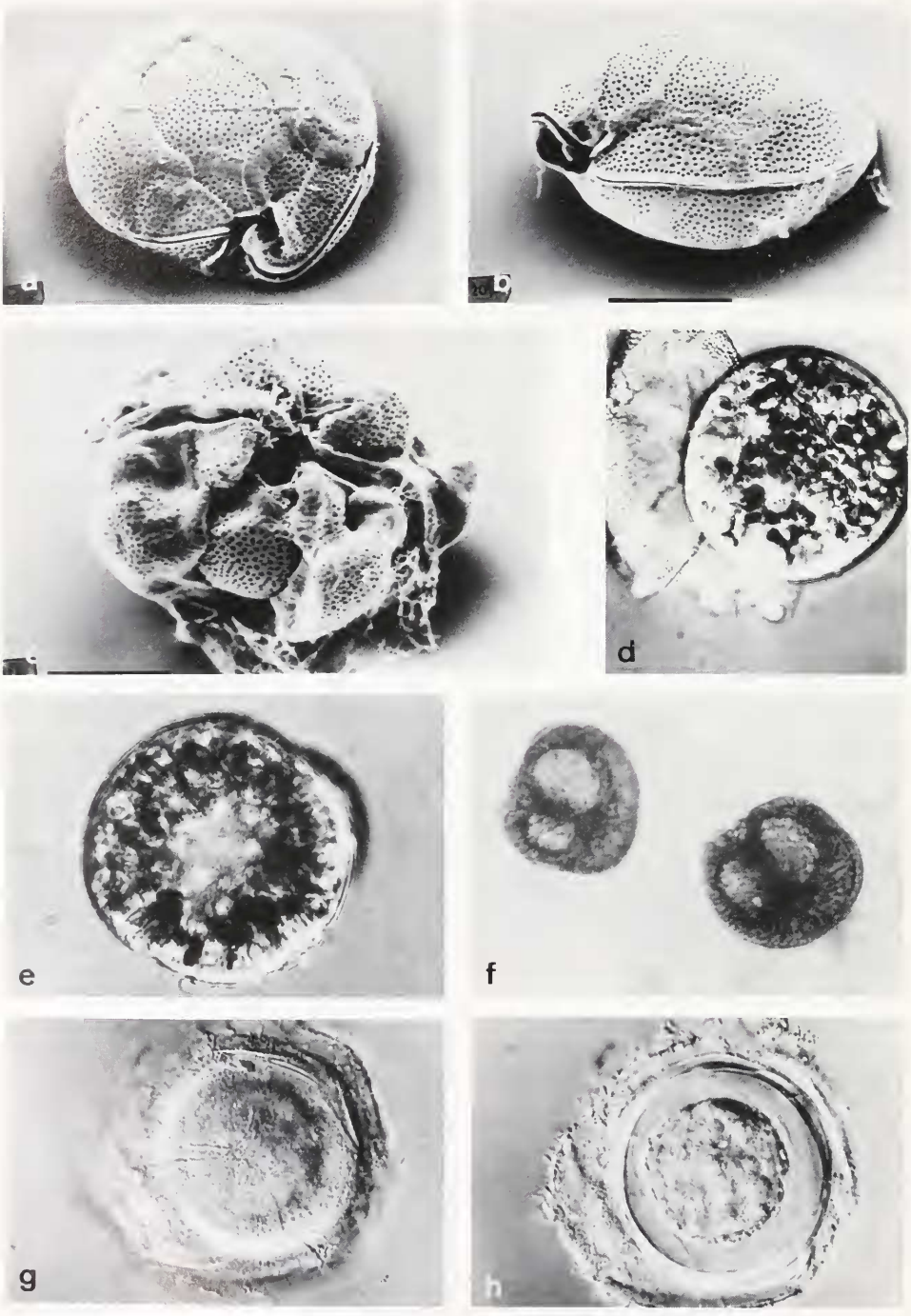
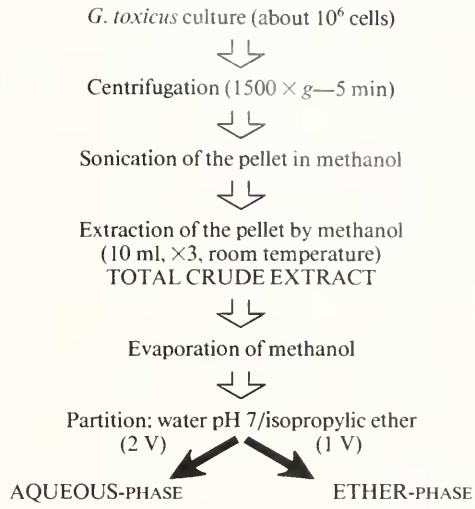



FIGURE 4. (a, b) Typical *G. toxicus* cells, SEM. The black line represents 20 $\mu$m (Durand and Coute photographs). (c) A deformed *G. toxicus* cell, SEM. The black line represents 20 $\mu$m. (d) Cell during ecdysis, 600×. (e) Aspect of a small, apparently naked cell, 900×. (f) Two typical cells after small dehydration process, 350×. (g, h) Aspect of a "temporary cyst" of *G. toxicus* (2 different foci), 600×.

TABLE I

*Toxin extraction procedure for* Gambierdiscus toxicus *cultures*




assays resulted in the protocol outlined in Table I. The principal modifications were: (1) using centrifugation, instead of filtration on paper filters, for culture harvesting and cell sonication, which permitted us to use small amounts of solvent; and (2) extracting with "cold" (room temperature) methanol and partitioning with isopropylic ether. This method is simpler, more rapid, and enhanced the yield of toxin two- to fourfold.

To simplify the evaluation of MLD for the initial methanol extract and to save mice, we established a relationship between the cell-pellet weight and the corresponding cell number (calculated using a Coulter counter). The equation of the linear relationship is:

$$Y = 0.056 + 0.263X$$

(Y represents the cell number: X represents the weight; n = 48 samples tested). Thus, we established that one million *G. toxicus* cells weighed 0.36 g.

*Results*

For toxicity screening we extracted many batches of *G. toxicus* cultivated in different media conditions. We also looked for differences between clonal and non-clonal strains. For these experiments cultures were harvested when they reached the stationary growth phase. Results are given in Table II.

Analysis of the data shows that differences between toxicities for various culture conditions were not significant. Other preliminary assays suggest that bacteria have little influence on total toxin production of *G. toxicus,* as the same toxicities were found for contaminated cultures (prior to antibiotic treatment) and "axenized" cultures (less than one bacteria per dinoflagellate cell) (Durand, 1987). In addition, we did not find a large increase in toxicity in aged cultures, in contrast to what other workers reported (Yasumoto, *et al.,* 1979; Bergmann and Alam, 1981).

TABLE II

*Compilation of* Gambierdiscus toxicus *toxicity data*

| Strain | Culture medium | Cell number ($10^3$) | Total crude extract | | Toxicity % | |
|---|---|---|---|---|---|---|
| | | | Total MU | Cells/MU | Ether-phase | Aqueous-phase |
| Clone 1 | MPP | 539 | 800 | 680 | ND | ND |
| | MS + MPP | 324 | 625 | 520 | 8 | 72 |
| | | 288 | 290 | 1000 | 34 | ND |
| Clone 2 | MS + MPP | 550 | 550 | 1000 | 18 | 82 |
| | | 218 | 180 | 1220 | 3 | ND |
| | LATEUR | 421 | 190 | 2240 | 8 | ND |
| | | 1843 | 1860 | 980 | ND | ND |
| Clone 3 | MPP | 307 | 340 | 900 | 3 | 56 |
| | | 205 | 180 | 1140 | 4 | ND |
| | MS | 203 | 150 | 1360 | 10 | 83 |
| | MS + MPP | 1500 | 1700 | 880 | 12 | 88 |
| | | 769 | 750 | 1020 | ND | ND |
| Clone 4 | MS + MPP | 243 | 235 | 1040 | 11 | 60 |
| | | 1640 | 700 | 2340 | 28 | 71 |
| Clone 5 | MPP | 326 | 400 | 820 | ND | ND |
| | | 400 | 625 | 640 | 19 | 74 |
| | MS | 336 | 225 | 1500 | ND | ND |
| | MS + MPP | 1040 | 420 | 2480 | ND | ND |
| | | 166 | 170 | 980 | ND | ND |
| Strain A | MPP | 726 | 480 | 1520 | ND | ND |
| | MS + MPP | 568 | 470 | 1200 | 13 | 74 |
| | | 2200 | 1050 | 2100 | 18 | 81 |
| Strain B | MPP | 1486 | 1560 | 960 | 13 | 32 |
| | | 2015 | 4075 | 500 | 21 | 54 |
| | MS + MPP | 727 | 725 | 1000 | ND | ND |

ND: non-determined; MU: mouse unit [equals Minimal Lethal Dose (MLD) ÷ 50]; MPP: culturing medium (Provasoli, 1958); MS: Shepard's artificial medium (Shepard, 1968).

The mean lethality for our *G. toxicus* strains was 1200 cells/MU.

$10^6$ *G. toxicus* = 0.36 g = 800 MU; MLD = 1–3 mg/kg.

We concluded that, under laboratory culture conditions, *G. toxicus* remained very toxic—in the same range that was reported for wild populations (taking into account the extraction yield by Yasumoto *et al.*, 1979, and Bagnis *et al.*, 1980).

*CTX-like and MTX-like toxicities*

During the toxin extraction procedure, the partition step always resulted in two toxic fractions: a water-soluble fraction (MTX-like) and an ether-soluble fraction (CTX-like). These fractions appeared in a CTX-like/MTX-like ratio of about 0.2. Figure 5 gives the dose/time-to-death curve that was obtained with one CTX-like extract. It must be noted that the minimal death time for this toxic fraction is higher than that reported for ciguatoxin extracted from fish (Tachibana, 1980; Lewis and Endean, 1984). This suggests that the ether phase obtained from *G. toxicus* cultures

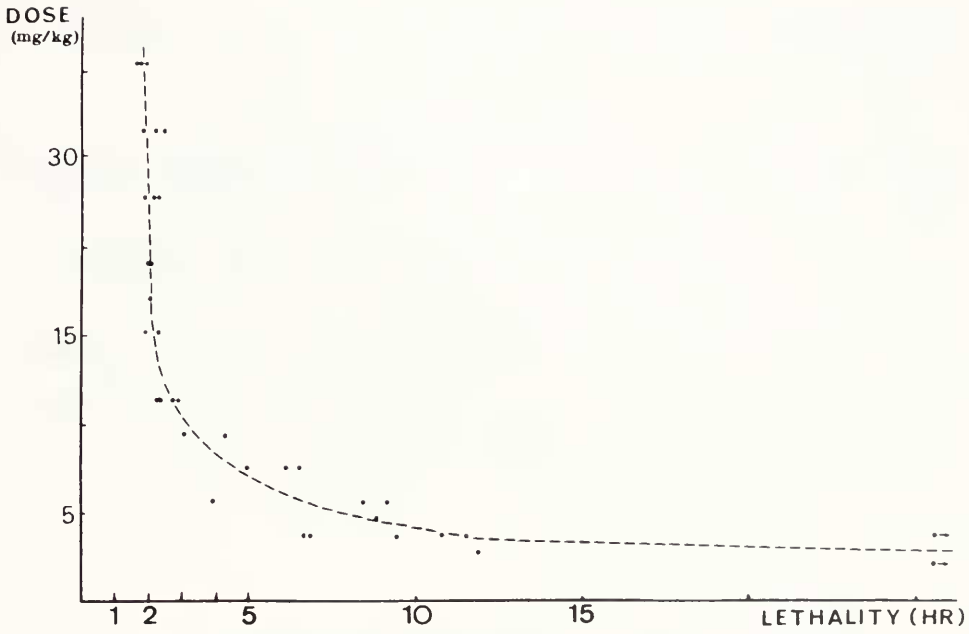



FIGURE 5. Relationship between dose and death-time for a crude ether phase from *Gambierdiscus toxicus* cultures (intraperitoneal injection in 20 g mice).

is not equivalent to the one obtained from fish. In particular, it could contain MTX (as already suggested by Yasumoto *et al.*, 1979), CTX-MTX complex, or another toxic compound. Further studies are necessary to characterize the toxin content of *G. toxicus*.

## CONCLUSION

We obtained some stable, well-defined uni-algal cultures of *G. toxicus* (different clones, in different media). Development of large-scale culturing must, at present, take into account the benthic behavior of this dinoflagellate, and should employ similar techniques as those used for animal-cell cultures.

*G. toxicus* in culture remains very toxic, thus it could be a superior raw material for providing cleaner and more abundant amounts of toxins: certainly maitotoxin and perhaps ciguatoxin, if further toxin characterization confirms its presence in the ether phase. The relationship between the two toxins remains to be elucidated, as does the importance of *G. toxicus* in ciguatera outbreaks. As this dinoflagellate is the focus of several studies, we suggest a standardization of its toxicity evaluation in order to help comparisons.

## ACKNOWLEDGMENTS

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